

CLAIMS

1. A polypeptide having the following physicochemical properties (1) to (5):

5 (1) Action: It asymmetrically reduces N-benzyl-3-pyrrolidinone to produce (S)-N-benzyl-3-pyrrolidinol with NADPH as a coenzyme;

(2) Optimum action pH: 4.5 to 5.5;

(3) Optimum action temperature: 40°C to 45°C;

10 (4) Molecular weight: About 29,000 as determined by gel filtration analysis, about 35,000 as determined by SDS-polyacrylamide gel electrophoresis analysis;

(5) Inhibitor: It is inhibited by the divalent copper ion.

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2. A polypeptide described in the following (a) or (b):

(a) A polypeptide having the amino acid sequence shown under SEQ ID NO:1 in the sequence listing;

20 (b) A polypeptide having an amino acid sequence obtainable from the amino acid sequence shown under SEQ ID NO:1 in the sequence listing by substitution, insertion, deletion and/or addition of one or more amino acids and having enzyme activity in asymmetrically reducing N-benzyl-3-pyrrolidinone to produce (S)-N-benzyl-3-pyrrolidinol.

25 3. The polypeptide according to Claim 1 or 2 which is derived from a microorganism belonging to the genus Micrococcus.

30 4. The polypeptide according to Claim 3, wherein said microorganism is the strain Micrococcus

luteus IFO 13867.

5. A DNA coding for the polypeptide according to any of Claims 1 to 4.

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6. A DNA

coding for a polypeptide having enzyme activity in asymmetrically reducing N-benzyl-3-pyrrolidinone to produce (S)-N-benzyl-3-pyrrolidinol, and

10 hybridizing with a DNA having a nucleotide sequence shown under SEQ ID NO:2 in the sequence listing under stringent conditions.

15 7. A DNA

coding for a polypeptide having enzyme activity in asymmetrically reducing N-benzyl-3-pyrrolidinone to produce (S)-N-benzyl-3-pyrrolidinol, and

20 having at least 60% sequence identity with a nucleotide sequence shown under SEQ ID NO:2 in the sequence listing.

8. An expression vector containing DNAs according to any of Claims 5 to 7.

25 9. The expression vector according to Claim 8, which is a plasmid pTSBH.

10. The expression vector according to Claim 8, which contains a DNA coding for a polypeptide having 30 glucose dehydrogenase activity.

11. The expression vector according to Claim 10, wherein said polypeptide having glucose dehydrogenase

activity is a Bacillus megaterium-derived glucose dehydrogenase.

12. The expression vector according to Claim 11,
5 which is a plasmid pTSBG1.

13. A transformant containing the expression vector according to any of Claims 8 to 12.

10 14. A transformant containing both the expression vector according to Claim 8 or 9 and an expression vector containing a DNA coding for a polypeptide having glucose dehydrogenase activity.

15 15. The transformant according to Claim 14, wherein said polypeptide having glucose dehydrogenase activity is a Bacillus megaterium-derived glucose dehydrogenase.

20 16. The transformant according to any of Claims 13 to 15,
wherein a host thereof is Escherichia coli.

25 17. The transformant according to Claim 16,
which is Escherichia coli HB101 (pTSBH).

18. The transformant according to Claim 16,
which is Escherichia coli HB101 (pTSBG1).

30 19. The transformant according to Claim 16,
which is Escherichia coli HB101 (pTSBH, pSTVG).

20. A production method of (S)-N-benzyl-3-

pyrrolidinol comprising

a step of reacting the transformant according to any of Claims 13 to 19 and/or a treated product thereof with N-benzyl-3-pyrrolidinone, and

5 a step of harvesting the thus-produced (S)-N-benzyl-3-pyrrolidinol.

21. The method according to Claim 20,
wherein the step of reacting is carried out in the
10 presence of a coenzyme regenerating system.